NaCI	125 mmol/L
KCI	4.5 mmol/L
$NaHCO_3$	200 mg/L

REMARKS

By the present Preliminary Amendment claims 11-16 has been added.

It is respectfully submitted that the claims now pending in this application are patentably distinct over Pözschke' 1992 (the inventor herein is a co-author of the Pözschke' 1992 article).

The Examiner asserts that Pötzschke' 1992 teaches the separation of cross-linked hemoglobin molecules into different fractions. This is incorrect. The difference between the claimed invention and Pötzschke consists in that in the instant application there is a **preparative** fractionation of cross-linked hemoglobin molecules according to their molecular weights, so that fractions are obtained, which contain **re-useable** amounts of molecularly uniform hemoglobin polymers. Contrary to that, Pötzschke describes the **analytical** determination of distributions of molecular sizes

(hydrodynamic volumes) of cross-linked hemoglobin molecules. The difference is mainly based on the following points leading to a non-transformability of the Pötzschke method:

a) Molecular sizes are only proportional to the molecular weights, when the molecular structures are similar. In 1992, when the reference Pötzschke was published, no one in the world knew the molecular structure of hemoglobin polymers in aqueous electrolyte solution (the Pötzschke reference is silent thereabout). Consequently, the reference of Pötzschke from 1992 cannot disclose fractionation of hemoglobin polymers with gel-permeation chromatography according to polymers molecular weights. The so-called determinations of molecular weights mentioned in the reference are estimations for the molecular weights. The molecular structure in solution of hemoglobin polymers was cleared up later, and published in Pötzschke, et al., Art. cells, Blood Subs., Immob. Biotech. 25 (1997): 527 540, (copy of the article is enclosed together with an Information Disclosure Statement). The main result of that 1997 reference is, that the structure-in-solution of the hemoglobin polymers is on the one hand similar at all respective molecular weights, but on the other hand different from that of native globular proteins. Only in 1996, 1997 it has become known that molecular size fractions of hemoglobin

polymers obtained with gel-permeation chromatography are molecular weight fractions which shows that this cannot have been known in 1992. Furthermore, although the hydrodynamic volume fractions are molecular weight fractions, the "molecular weights" of hemoglobin polymers given in Pötzschke are, in part extremely wrong since they have been made in accordance with native proteins, but not in accordance with the correct structures-in-solution, as it has been found out later (please see page 534 in the 1997 reference, where it is pointed out that the usual calibration method for determining molecular weight by chromatography was not accurate, which inaccurate method however having been used by Pötzschke' 1992, see page 288, curve D and page 533, curves A-C in the enclosed 1997 reference: the error is already about 130% at a degree of polymerisation of only 10, and about 240% at a degree of polymerisation of 30). Therefore, reference Pötzschke' 1992 does not disclose fractionating hemoglobin polymers according to their molecular weights as it has been done by the present invention.

b) Analytical use of a substance separating method by definition means, that the fractionated molecules are only used to act - to avoid disturbances best directly after arising - as objects for a substance detector. To save

analytical material, analytical methods are normally built up in the smallest possible extension. Thus, the substance fractions formed by the analytical method during work are not separated fractions, **further useable** for other purposes (contrary to the present fractions). Moreover scale up of an analytical method to a distinct larger preparative scale is often accompanied by a loss of performance and, in some cases, scale up therefore is impossible. The degree of scale-up which is possible is not foreseeable, but must be determined experimentally.

It should be pointed out that Pötzschke' 1992 is connected with a new artificial oxygen transporter which is stable due to the specific cross-linking method. Because the cross-linked hemoglobins are stable, they can be purified by ultrafiltration see at page 290, under "Results" where it is pointed out that: "In addition, stabilized hemoglobin hyperpolymers were well fractionable in ultrafiltration (not documented here)". That means that the Pötzschke reference is not concerned with a purification of the cross-linked hemoglobin molecules by using Sephacryl S-400 gel as stated in the office action. Furthermore, from the cited paragraph "Results" at page 290 the following may be taken:

In this paragraph it is pointed out that the reaction of the hemoglobin with a specific cross-linking agent did lead to hemoglobin hyperpolymers which did not show any changes in molecular size distribution as determined by size exclusion chromatography, as can be seen in figure 1A and figures 1B. When looking at figure 1A and 1B shown at page 288, it can be seen that these figures show the original gelchromatograms of a mixture of hyperpolymers of cross-linked hemoglobin and non-crosslinked hemoglobin made from different incubation times (see the description of the figures beneath the figures at page 288). As can clearly be taken therefrom as well as from the respective paragraph at page 290 ("Results"), it can be seen that there is no **fractionation** of the hemoglobin cross-linked molecules but only a chromatogram which shows that molecular sizes are broadly distributed.

As it has already been pointed out Pötzschke does not separate the cross-linked hemoglobin molecules with regard to their molecular weights by the aid of exclusion molecular chromatography as presently carried out. The claimed method does separate the molecules of a sólved compound in accordance with its hydrodynamic volume (V_n). That volume is correlated with the molecular weight (M) by the intrinsic viscosity (V_n is proportional M x $[\eta]$, η = intrinsic viscosity.

Consequently, when molecular weight fractions are desired from a mixture of polymers with a broad molecular size distribution, each fraction must have similar intrinsic viscosities (of the molecules). If this is not the case, molecules with similar molecular weight will be eluted into different molecular size fractions, although belonging to the same molecular weight fraction.

However during a cross-linking reaction with hemoglobin, compounds may be obtained which are cross-linked in a different way. The cross-linking factor however is responsible for the intrinsic viscosity of the respective molecule. With the present method, it has surprisingly been found out that such cross-linked hemoglobin compounds with specific intrinsic viscosities are separated into fractions of different molecular weights because the intrinsic viscosity of the respective molecular weight fractions are very similar. In view of the disclosure of the reference, this could not be expected since the Pötzschke reference does only disclose that a cross-linking reaction between hemoglobin and specific cross-linking agents lead to stabilized hemoglobin hyperpolymers which show a broad size distribution. Since according to the Pötzschke method as disclosed a separation with Sephacryl has not been obtained even with small amounts,

it was highly surprising that using higher amounts of starting materials to be separated did lead to such a separation.

It is to be noted that figures A to D at page 288 of Pötzschke' 1992 clearly show the distribution of the crosslinked molecules obtained which is shown with reference to their size exclusion volume, see also the explanations at the first paragraph of page 290 of Pötzschke' 1992. This clearly shows that the material obtained after the cross-linking reaction comprises a broad distribution of molecular sizes shown by the exclusion volumes of figures A to D which reflect the gelchromatograms of the method of the cited reference which in view of this result can in no case be a purification or even a separation of the molecules into different molecular weight fractions, as explained above.

In summary, Pötzschke does not describe a preparative fractionation of crosslinked hemoglobin.

In view of the above, it is respectfully submitted that the presently pending claims are neither anticipated by nor are they obvious over Pötzschke' 1992.

Applicant further respectfully submits that the pending claims would not be obvious over Pötzschke' 1992 in view of Bonhard.

With regard to Bonhard the following should be pointed out. The aim of the present invention is to separate fractions of molecularly uniform hemoglobin polymers. Starting material for the present invention are not the native hemoglobins from which the hemoglobin polyers are synthesized by crosslinking (such syntheses are not subject of the present invention), but already crosslinked materials (pure mixtures of hemoglobin polymers without any un-cross-linked hemoglobin solved in aqueous electrolytes probably such as obtained as final product according to the bonhard reference) which are then treated according to the present invention to obtain the molecularly uniform hyperpolymeric hemoglobins. Furthermore, in analyolgy, the present final product is not the hemoglobin cross-linked with glutaraldehyde, but molecularly uniform hyperpolymeric hemoglobins (cross-linked with glutaraldehyde). Thus, the method according to the Bonhard reference uses a different starting material (row mixture or synthesis products of hemoglobin, cross-linked with glutaraldehyde), has another aim (to remove monomeric, not polymerized hemoglobin) and another final product (a mixture of pure hemoglobin polymers,

which could be used as starting material in the presently claim method). The material to be removed in the Bonhard reference (monomeric, not polymerized hemoglobin) is a globular protein, its physico-chemical properties are distinct from that of hemoglobin polymers. Thus, the Examiner's assertion that presently and according to the Bonhard reference the same starting material, namely non-crosslinked hemoglobin, would be used is not correct since presently only a method for the preparation of molecularly uniform hyperpolymeric hemoglobins is claimed, but not for preparing crosslinked hemoglobin. Therefore, appellant respectfully submits that the Examiner's argument of *prima facie* obviousness cannot be correct since besides any similar or non-similar motivation, there is a completely different method according to the claim 6 at least when considering Bonhard. Moreover, when looking through the Bonhard reference, it can be seen that in view of the known separation of non-cross-linked hemoglobin having a completely different structure from the very highly polymer hyperhemoglobins and, therefore, having completely different properties of their physical and chemical behavior, it could not have been expected that an agent which is said to be capable of separating two completely different compounds, could also be used to separate compounds which are chemically and physically similar in their behavior

such as the hyperpolmer hemoglobins of different molecular weights. Insofar and contrary to the Examiner's position, one skilled in the art would not at all have had any motivation to use ammonium sulfate in particular taken into account that at the time the present invention was conceived, one of ordinary skill in the art had no knowledge about the physical behavior of then a new group of chemical compounds such as hyperpolymeric hemoglobins.

In view of the above, it is respectfully submitted that all of the claims now pending in the application patentably define over the prior art and are, therefore, allowable.

CONCLUSION

In view of the foregoing, it is respectfully submitted that the application is in condition for allowance, and allowance of the application is respectfully requested.

Should the Examiner require or consider it advisable that the specification, claims and/or drawings be further amended or corrected in formal respects, in order to place the case in condition for final allowance, then it is respectfully requested that such amendment or correction be carried out by

Examiner's Amendment and the case passed to issue. Alternatively, should the Examiner feel that a personal discussion might be helpful in advancing this case to allowance, the Examiner is invited to Telephone the undersigned.

Respectfully submitted
for applicant,

David Toren
Reg. No. 19,468

Dated: February 8, 1999

AKO-TOREN
1251 Avenue of the Americas
New York, New York 10020-1182
(212) 278-1000